Labeling of Poly(lactide-co-glycolide) Hydroxyl Groups with Naphthyl Isocyanate: Analysis and Limitations

J. Garner[1], Justin Hadar[1], S. Skidmore[1], H. Park[1], K. Park[1], Y. Wang[2], C. Plavchak[2], W. Smith[3], Y. Jhon[3], D. Zhang[2], Y. Zou[2]
[1] Akina, Inc., West Lafayette, IN 47906, U.S.A.
[2] Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD 20993, U.S.A.
[3] Office of Pharmaceutical Quality Research, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD 20993, U.S.A
jg@akinainc.com

Introduction

Poly(lactide-co-glycolide), commonly known as poly(lactic-co-glycolic acid) (PLGA), used in drug-delivery systems, can be manufactured in a variety of molecular structures, including linear and star-shaped polymers, by controlling the type of polymerization initiator. Since star-shaped polymers have a higher number of hydroxyl endcaps per molecular weight unit, a potential method for analyzing molecular structure is to conjugate a dye label to the hydroxyl endcap to increase the polymers' UV absorbance in respect to the number of hydroxyls available for attachment.

Methods

The micromolar (µM) content of hydroxyl group per gram of a series of polymer standards with known molecular weight and branching degree [1] was calculated by multiplying the branching number by 1 gram divided by the number average molecular weight (moles polymer in 1g) and multiplying by 10^6. The polymers were reacted with napthyl isocyanate (NPI) in dichloromethane for 16 - 24 hours at 37 °C in the presence of stannous octanoate catalyst. Subsequently purified by filtration and precipitation, these polymers were analyzed by Gel Permeation Chromatography (GPC) with an Ultraviolet (UV) detector in acetonitrile to monitor absorbance changes at the dye wavelength. Polymers were characterized by proton nuclear magnetic resonance (HNMR) and GPC to confirm reaction and characterize changes in molecular weight.

Results

The GPC-UV chromatograms were used to calculate the percent area of the absorbance at 290 nm (NPI dye) relative to the peak area of the absorbance at 200 nm (overall content), both absorbance values corrected to subtract the contributions of dye and polymer to the other absorbance value. A strong correlation between the calculated µM/g hydroxyl content and the NPI dye content was observed (**Figure 1**). The relationship of increased NPI conjugation in response to hydroxyl content was also confirmed by HNMR (**Figure 2**). Despite this, a strong reduction in molecular weight for a wide range of polymers during the reaction was observed (**Table 1**).

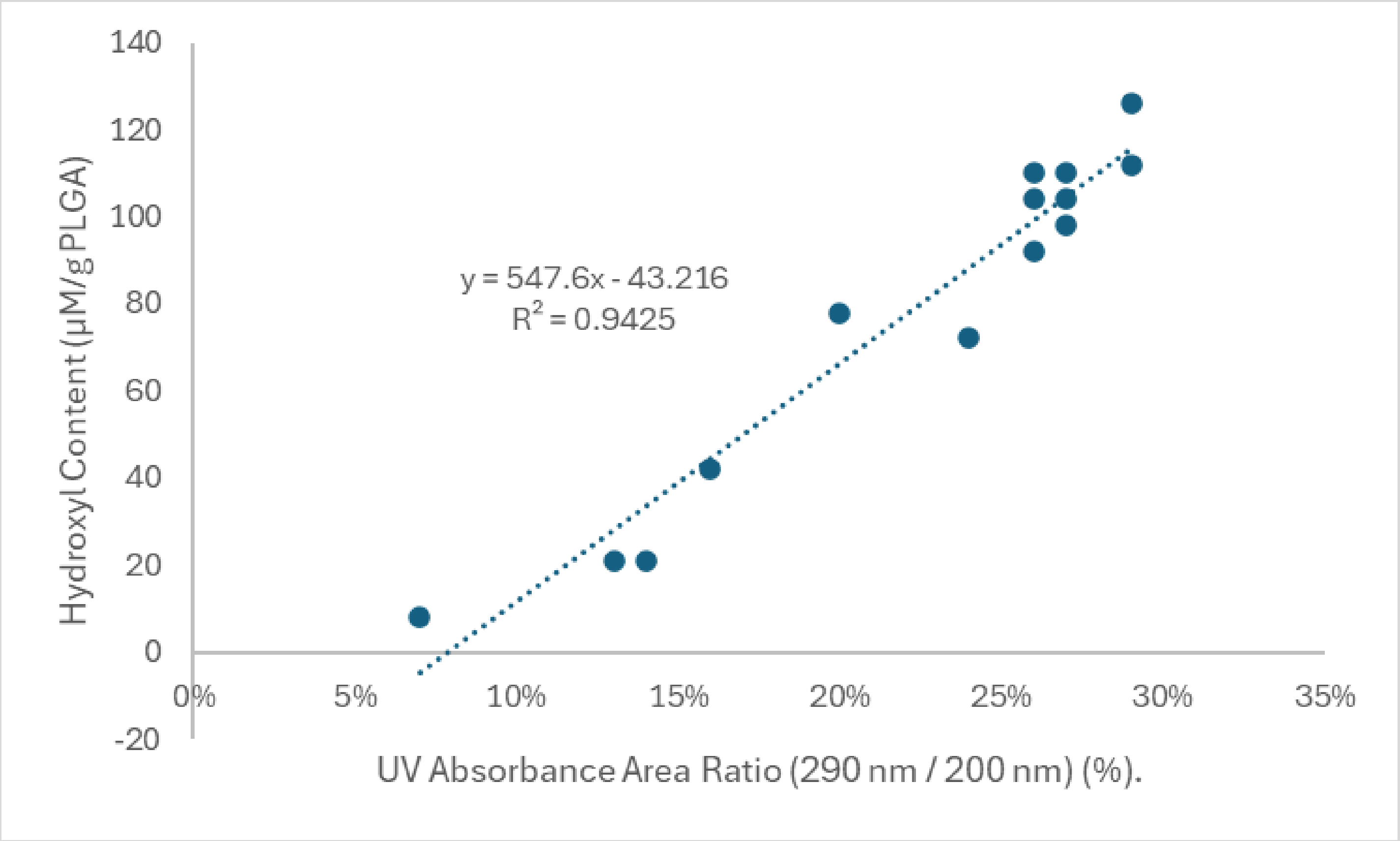

Figure 1. GPC-UV/Vis determination of NPI-conjugation relationship to micromolar hydroxyl units per gram PLGA.

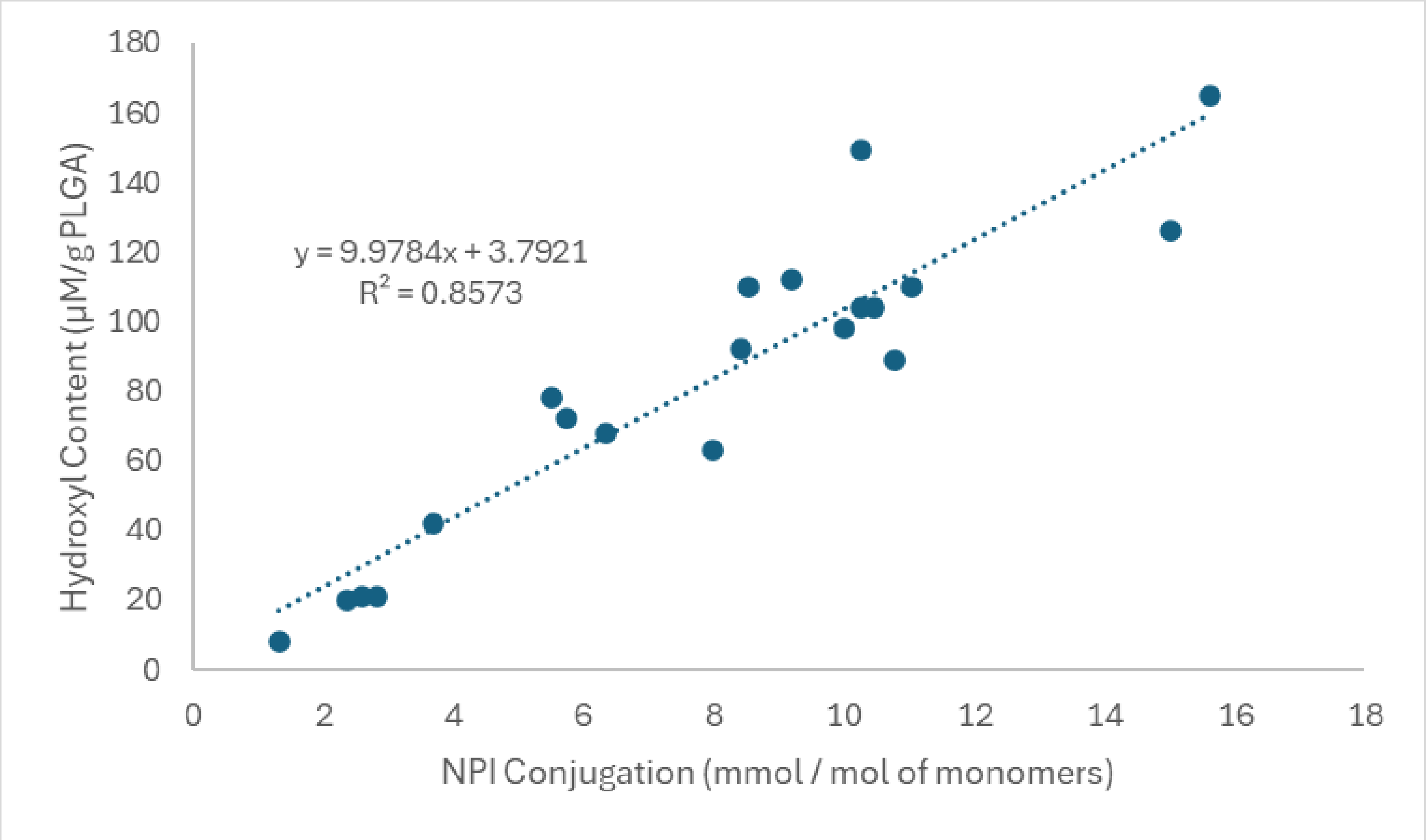

Figure 2. HNMR determination of NPI-conjugation relationship to micromolar hydroxyl units per gram PLGA.

Conclusion

Dozens of hydroxyl-dye conjugation reactions were explored using a variety of dyes. Among them, NPI showed the greatest promise for quantitative determination of hydroxyl content in PLGA. Unlike carboxylic acid conjugation, which can be achieved under gentle conditions [2], the weak hydroxyl nucleophile requires a more robust set of reaction conditions. These conditions, however, also lend themselves to side reactions such as potential lysis of the PLGA molecule [3]. These findings highlight a challenge which limits the applicability of dye-endcap labelling towards determination of star-PLGA presence which necessitates alternative, GPC-4D techniques.

Table 1. PLGAs used in the study with calculated µM/g hydroxyl content, LA:GA ratio (HNMR), napthyl conjugation (mM NPI/Mole LA+GA, HNMR), and number average molecular weight before and after NPI reaction. Molecular weights were measured using GPC with external standards (GPC-ES).

PLGA (lot#)	LA:GA (NMR)	µM Hydroxyl/g PLGA	HNMR NPI (mM/Mole LA+GA)	Mn (GPC-ES) (*Pre-Reaction NPI*)	Mn (GPC-ES) (*Post reaction with NPI*) (% change)
AP001 240425RAI-A	100:0	92	8.4	14,011	11,901 (-15%)
AP018 180601RAI-A	77:23	20	2.4	50,977	35,083 (-31%)
AP040 230811TMT-A	49:51	42	3.7	23,576	11,439 (-51%)
AP075 181206FAJ-A	85:15	8	1.3	123,622	54,529 (-56%)
AP083 190327FAJ-A	59:41	21	2.8	46,562	20,169 (-57%)
AP092 181029NDR-A	100:0	165	15.6	18,128	14,895 (-18%)
AP227 230223RAI-A	55:45	98	10.0	40,709	22,206 (-45%)
AP228 191003RAI-B	55:45	112	9.2	53,494	35,576 (-33%)
AP229 230228RAI-A	55:45	72	5.7	41,569	30,248 (-27%)
AP229 190628RAI-A	55:45	68	6.3	44,167	28,247 (-36%)
AP233 180220JMB-A	55:45	21	2.6	48,077	24,560 (-49%)
AP236 190710RAI-A	54:46	110	8.5	45,324	34,417 (-24%)
AP237 180717FAJ-A	75:25	63	8.0	47,586	31,411 (-34%)
AP241 180719FAJ-A	75:25	89	10.8	45,080	18,579 (-59%)
AP242 180723FAJ-A	73:27	104	10.3	57,568	52,100 (-9%)
AP278 210713RAI-B	52:48	149	10.3	26,926	20,787 (-23%)
CB001 230617CBP-442	51:49	110	11.0	9084	6,485 (-29%)
CB002 210817CBP-161	51.49	104	10.5	9622	8,384 (-13%)
CB005 230122CBP-657	74:26	126	15.0	7967	5,883 (-26%)
CB006 210616CBP-673	55:45	78	5.5	28,321	15,089 (-47%)

[1] Hadar, Justin, et al. "Method matters: Development of characterization techniques for branched and glucose-poly(lactide-co-glycolide) polymers." Journal of Controlled Release 320 (2020): 484-494.

[2] Garner, John, et al. "A New Analytical Method for Quantifying Acid-End-Cap PLGA in Sub-Milligram Quantities." Molecular Pharmaceutics 22.1 (2024): 446-458.

[3] Rekker, R. F., and W. Th. Nauta. "The reaction of hydroxy-esters and amino-esters with isocyanates." Recueil des Travaux Chimiques des Pays-Bas 83.10 (1964): 1039-1046.

Acknowledgements

This work was supported by the Broad Agency Announcement (BAA) Contract # 75F40123C00192 from the U.S. Food and Drug Administration (FDA). The content is solely the responsibility of the authors and does not necessarily represent the official views or policies of the U.S. FDA.